PILOT SCALE CONVERSION OF CELLULOSE TO ETHANOL

Dana K. Becker, Paul J. Blotkamp, George H. Emert

Biomass Research Center
University of Arkansas
415 Administration Building
Fayetteville, Arkansas

INTRODUCTION

Interest in cellulose as a renewable source of alcohol fuels and other chemicals has increased as the price of petroleum products continues to rise. Extensive research has been conducted in the area of cellulose utilization for a number of years (1, 2, 3, 4, 5). However, with the exception of The U. S. Army Natick Research Command which has operated a prepilot program for the enzymatic conversion of cellulose to glucose since 1976 (6, 7), these investigations have been confined to the laboratory.

The importance of piloting a complete process for the conversion of cellulose to ethanol was recognized by this laboratory in 1974. The complexity of combining the material handling of bulky slurries such as air classified municipal solid waste (MSW) and pulp mill waste (PMW) with the aseptic operation of an enzyme production facility posed a unique set of problems which could not adequately be addressed on a laboratory scale. In order to address these problems, it was believed that the design of a pilot plant should include the flexibility of handling feedstocks of widely varying composition and moisture content. Operation of a pilot plant would allow the identification and testing of equipment for the preparation and transfer of slurries, sterilization, and liquid/solid separation.

The economic feasibility of a capital intensive process such as the cellulose to ethanol process requires that the use of highly specialized exotic equipment be kept to a minimum. As a result of this, low cost chemical reactors would be evaluated as fermentation vessels. The vessels first tested as "off the shelf items" could then be modified as necessary to accommodate the individual requirements of each set of fermentation conditions. In this way parameters such as agitation, aeration, temperature and pH control, and sterility could be evaluated and adjusted as needed. Using these criteria the biochemical conversion of cellulose to ethanol was scaled-up approximately 100 fold from 10L laboratory fermenters to 1000L vessels in a pilot facility capable of processing 1 ton per day of cellulosic feedstock.

METHODS AND MATERIALS

Three strains of yeast were used during the pilot investigations of simultaneous saccharification fermentation (SSF). (8, 9). These were *Saccharomyces cerevisiae* ATCC 4132, obtained from the American Type Culture Collection, Rockville, Maryland; *Candida brassicae* IFO 1664, obtained from the Institute for Fermentation, Osaka, Japan (2); and a strain of *Saccharomyces* obtained from Budweiser, Joplin, Missouri.

Stock cultures were stored on Difco YM agar slants at 4°C. Seed cultures of each yeast were prepared by the addition of a portion of a stock culture into a shake flask containing a medium shown in Table I.[1] Shake flasks were inculated at 28°C for 18 hours. The shake flask culture was used to inoculate a 130L fermenter made by Fermentation Design, Inc., containing 100L of the medium in Table II. This culture was incubated for 18 hours at 30°C, pH 5.0, with an agitation speed of 120 RPM. The yeast seed culture was harvested into sterilized 15 gallon aluminum barrels prior to use in SSF. If the yeast was not used immediately the barrels were stored in a cold room at 4°C for no longer than 48 hours.

The mold *Trichoderma reesei* QM 9414 was obtained from ATCC. This organism was grown on potato dextrose agar at 29°C until sporulation occurred. The spore plates were stored at 4°C until use. *T. reesei* seed cultures were prepared by inoculating shake flasks with a portion of a spore plate. The culture medium used in the shake flasks is shown in Table III. The 1 liter shake flasks were scaled-up to 100 liter fermenters. Physical parameters controlled in the fermenters were aeration at 0.5 v/v/m and agitation speed at 300 RPM (100L fermenter). The seed cultures were incubated for 24 hours and then harvested aseptically into 15 gallon aluminum barrels to be transported to the pilot facility where it was used as inoculum for enzyme production.

A 10% v/v inoculum was used for initiation of cellulase induction stage in both batch and continuous phases of enzyme production. The medium used in enzyme production is described in Table IV. Avicel PH 105, comparable to MSW in inducing cellulase enzymes, was chosen as a model substrate because of its ease of handling and uniformity. Avicel PH 105 was obtained from American Viscose Co., Division of FMC, Marcus Hook, Pennsylvania. The length of incubation of the culture depended on the mode of enzyme production being used. Batch enzyme production lasted 96 to 120 hours whereas continuous enzyme production had a residence time of 50 hours (D=.02). Batch SSFs were run for 24 hours unless experimental design dictated otherwise. Semi-continuous SSFs were run for 96 to 120 hours with the residence time varying from 24 hours to 48 hours. Three major types of feedstocks were used, 1) purified cellulose (Solka floc.), 2) PMW (digester rejects, primary sludges, and digester fines), 3) MSW. None of the feedstocks received any type of pretreatment before use in the SSFs. However, MSW was at times pasteurized depending on experimental conditions. The MSW used in the SSFs had been shredded so that it would pass a 4" screen and then air classified prior to arrival at the pilot plant.

Assays for measurement of enzyme activity and protein concentration were conducted as described by Blotkamp, et al (9). Glucose measurements were made with the use of a Yellow Springs Instrument Company Model 23A glucose analyzer. Total reducing sugars were measured by the dinitrosalicylic acid method (10). Ethanol was analyzed using a Perkin-Elmer Model 3920 B gas chromatograph or a Hewlett-Packard Model 5730 A gas chromatograph equipped with flame ionization

[1] Chemicals used in media formulations were mostly technical or reagent grade, however in the past year many of the compounds used were either fertilizer or food grade.

detectors, an electronic integrator, and a 6 ft. column of Porapak Q. Isothermal analysis was performed at 150°C.

Yeast populations were monitored by using dilution plating. Cellulose concentration of samples used in SSF was determined by using a modified version of the Van Soest procedures (11, 12). Moisture determinations were performed on an Ohaus moisture balance.

EQUIPMENT

The vessels used for enzyme production and SSF were 330 gal (1250 liter) capacity manufactured by Pfaudler (L/D=.78). Four of the five vessels were capable of aseptic operation. The vessels were constructed of stainless steel with carbon steel jackets. The vessels were fully jacketed for adequate temperature control and sterilization.

All process piping was stainless steel with welded connections except where piping entered the vessel. Flanged fittings with teflon gaskets were used at these points. No pumps were used as a precaution against contamination, the liquids and slurries were moved with pressure (sterile air or steam) or gravity. The agitator shafts were equipped with double mechanical seals filled with oil. Enzyme production vessels used two flat blade impellers, each having four blades (Di/Dt=.456). Agitation speed was 120 RPM, aeration was 0.5 v/v/m at which the k_La was 84 hr^{-1} vs 330 hr^{-1} on a lab scale (with water).

The baffle tray stripping column was constructed from 9" (I.D.) glass pipe with trays made of monel to resist corrosion. Associated process lines on the stripper were stainless steel. Pumps were used on the beer feed lines on the stripping column and recirculation loops to maintain solids in suspension.

A brief process flow diagram is presented in Fig. 1. After the enzyme production vessels were filled with nutrients and sterilized, the seed inoculum was transferred aseptically from the aluminum barrels to the vessels using nitrogen to pressurize the barrels. From this point the enzyme production could be run in either a batch or continuous mode. When enzyme was ready to be harvested a portion of the whole culture enzyme broth was transferred to the SSF vessel into which the cellulosic feedstock (PMW or MSW) would be added, along with the yeast. The SSF could be run in either batch or semi-continuous modes in which one half of material was transferred out every one half residence time. As the SSF was harvested the resulting beer slurry was moved to the beer storage tank where it could be pumped into the stripper column for ethanol recovery.

RESULTS

Enzyme Production

Performance of batch enzyme productions can be typified by the data presented in Figures 2 and 3. Relatively high levels of protein and β-*glucosidase* are present in the culture broth. These results compare favorably with those obtained in laboratory studies.

The pilot plant was modified to produce enzyme continuously in order to demonstrate feasibility on a large scale. The economical advantages of a

continuous process lie in reduced capital investment due to increased efficiency of vessel use. Results from continuous enzyme productions are shown in Figs. 4 and 5. From these graphs can be seen that the *β-glucosidase* is somewhat lower but the protein and FPRS remain almost as high as in batch culture. Use of the enzyme from batch as well as continuous enzyme production in small scale flask saccharification and SSFs indicate only small differences between the two enzyme preparations under the same conditions.

SIMULTANEOUS SACCHARIFICATION FERMENTATION

Batch SSFs were performed using a variety of substrates. Typical results for Solka floc. and pulp mill wastes are illustrated in Fig. 6 and Fig. 7 respectively. In both cases over 50% of the theoretical yield from cellulose to ethanol was achieved. Batch SSFs were run with cellulose concentrations ranging from 5 to 15%.

Semi-continuous SSFs utilized pulp mill wastes and municipal solid waste as primary feedstocks. Ethanol production can be seen in Fig. 8. Both MSW and PMW showed the same trend (Fig. 9) concerning ethanol yield, base utilization for pH control, and bacterial contaminant population. The presence of contaminants and increased base usage indicates the production of other acidic products. Lab scale continuous SSF operation has proved to be significantly better than batch SSF per unit time.

STRIPPING OPERATIONS

After the SSFs were completed the resultant beer slurry was pressured to the beer storage tank (Fig. 1). From the beer storage tank the slurry was pumped to the top of the baffle tray column (13) while steam was injected into the bottom of the column. As the beer slurry cascaded down the column the hot vapor from below contacted the descending liquid and effected the stripping of the ethanol from the beer feed. The column was designed to handle beer slurries with solids content as high as 10% and deliver a product stream of approximately 25% w/v ethanol from a feed containing 2.0 to 3.5% ethanol. The still bottoms ethanol concentration remained as low as 0.04%. In a large-scale plant the product from the slurry stripper will be rectified further to yield 95-100% industrial or motor grade ethanol as necessary.

DISCUSSION AND CONCLUSION

Many pieces of equipment used for materials handling were tested in the pilot plant. An example is a 750 gallon pulper which worked with some wood products but not very well with MSW because of the plastics and metal cans in the material. A rotary vacuum filter was used for dewatering some slurries but for the majority of feedstocks it was not acceptable. For these reasons the feedstocks used at the pilot plant, as outlined in this paper, received no pretreatment and were used in the process just as they were received.

The operation of the pilot plant in both a batch and continuous mode using potential industrial feedstocks demonstrated the enzymatic cellulose to ethanol technology on a substantially larger scale than had previously been reported. The size of the plant enabled the use of bulky materials, such as MSW, which was difficult on a laboratory scale. The results from the pilot plant enzyme

production compared very favorably with the laboratory results, however in the case of the SSFs the data from the pilot plant and the laboratory are only comparable for approximately the first 24 hours after which the pilot plant results lagged behind. For example, on batch SSFs that ran longer than 24 hours at the pilot plant the percent conversion to ethanol did not continue to rise as in the laboratory. With pulp mill wastes in laboratory studies, SSFs of 85 to 90% of theoretical conversion to ethanol was achieved in 48 hours compared to 55 to 60% conversion at the pilot plant. The reasons for the difference in results can be explained in part by the lack of adequate environmental controls such as temperature and pH due to poor heat and mass transfer in the high solids slurry of the SSFs. Contamination was also a problem in SSFs that ran for extended periods as evidenced by the increase in base utilization for pH control and the concomitant decrease in ethanol yields (Figs. 8, 9).

The data gathered from the operation of the pilot plant was used for extensive economic analysis of the cellulose to ethanol technology (14). The results of this analysis along with the problem areas mentioned above indicate further scale-up of the process from the 1 ton/day to a 50 ton/day facility should be carried out in order to identify specific equipment to be used on a commercial scale and execute process modifications toward enhancing the economic viability of the technology.

NOMENCLATURE

a	area
D	dilution rate
Di	impeller diameter
Dv	vessel diameter
I.D.	internal diameter
k_L	mass transfer coefficient
L	vessel length
m	minute
v	volume

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Table I

Yeast growth medium (flask)

	g/l
D -glucose	20.0
yeast extract	5.0
malt extract	5.0
bacto-peptone	5.0

Table II

Yeast growth medium (fermenter)

	g/l
D -glucose	20.5
$(NH_4)_2 SO_4$	1.5
$MgSO_4 \cdot 7H_2O$	0.11
$CaCl_2$	0.06
Cornsteep Liquor	7.5

Table III

T. reesei growth medium

	g/l
D -glucose	20.0
KH_2PO_4	2.0
$(NH_4)_2HPO_4$	1.23
$MgSO_4 \cdot 7H_2O$	1.0
$CaCl_2$	3.0
$FeSO_4$	0.05
$ZnSO_4$	0.014
$MnSO_4$	0.016
$CoCl_2$	0.04
$(NH_4)_2SO_4$	2.62
$(NH_2)_2CO$	1.7
Cornsteep	7.5

Table IV

T. reesei enzyme production medium

	g/l
Cellulose (Avicel 105)	20.0
KH_2PO_4	2.0
$(NH_4)_2HPO_4$	1.23
$MgSO_4 \cdot 7H_2O$	1.0
$CaCl_2$	3.0
$FeSO_4$	0.05
$ZnSO_4$	0.014
$MnSO_4$	0.016
$CoCl_2$	0.04
$(NH_4)_2 SO_4$	2.62
$(NH_2)_2 CO$	1.72
Cornsteep Liquor	7.5
Tween 80	0.2%

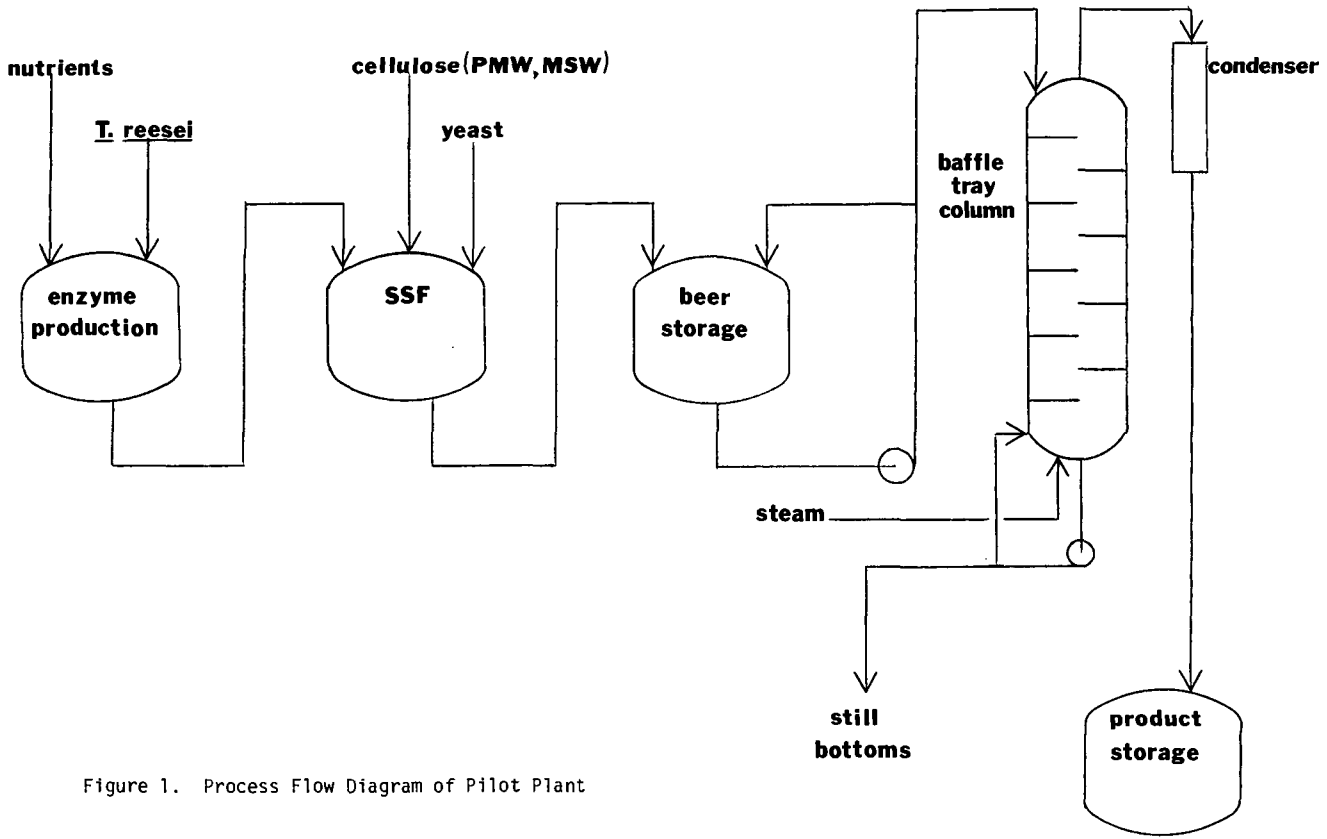

Figure 1. Process Flow Diagram of Pilot Plant

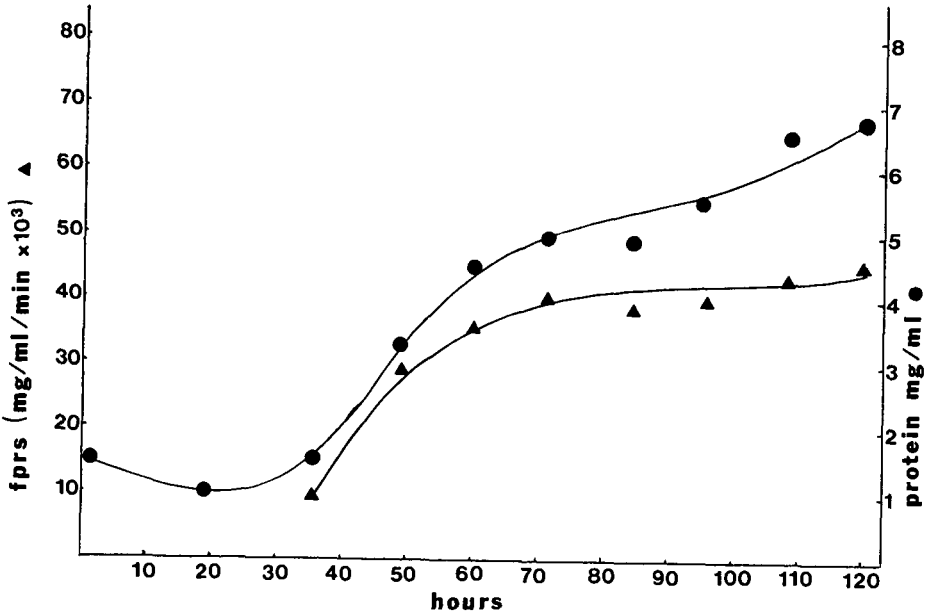

Figure 2. Batch Enzyme Production FPRS Activity and Protein Concentration

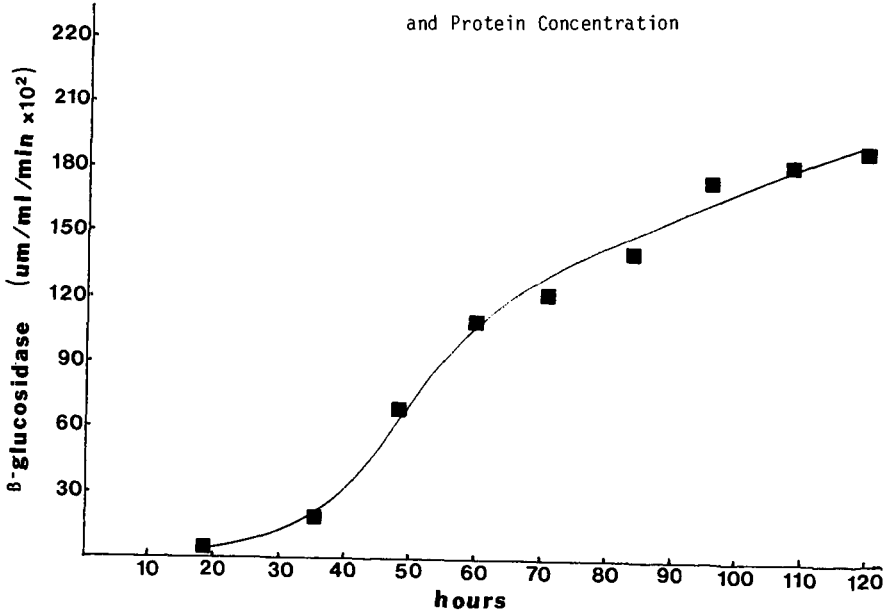

Figure 3. Batch enzyme production β-*glucosidase* Activity

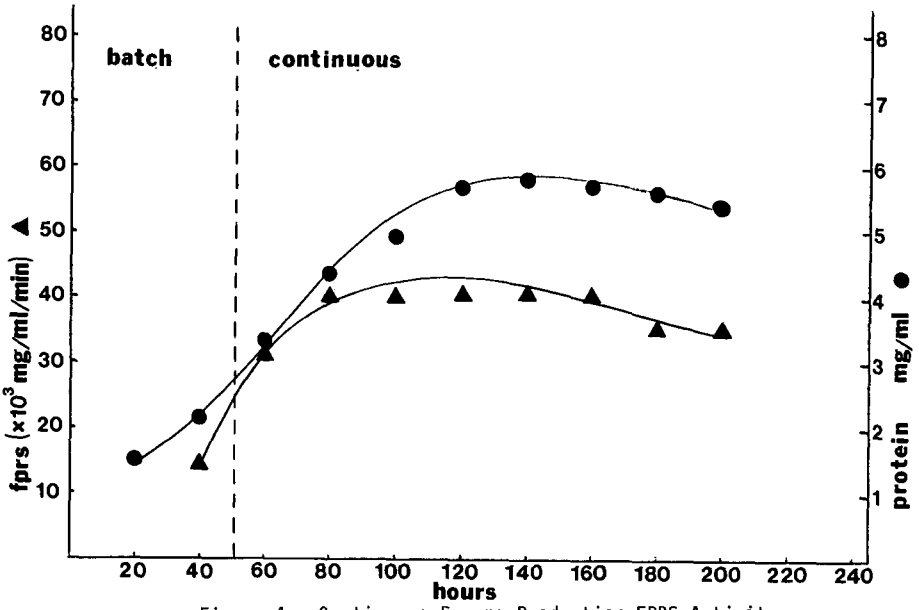

Figure 4. Continuous Enzyme Production FPRS Activity and Protein Concentration

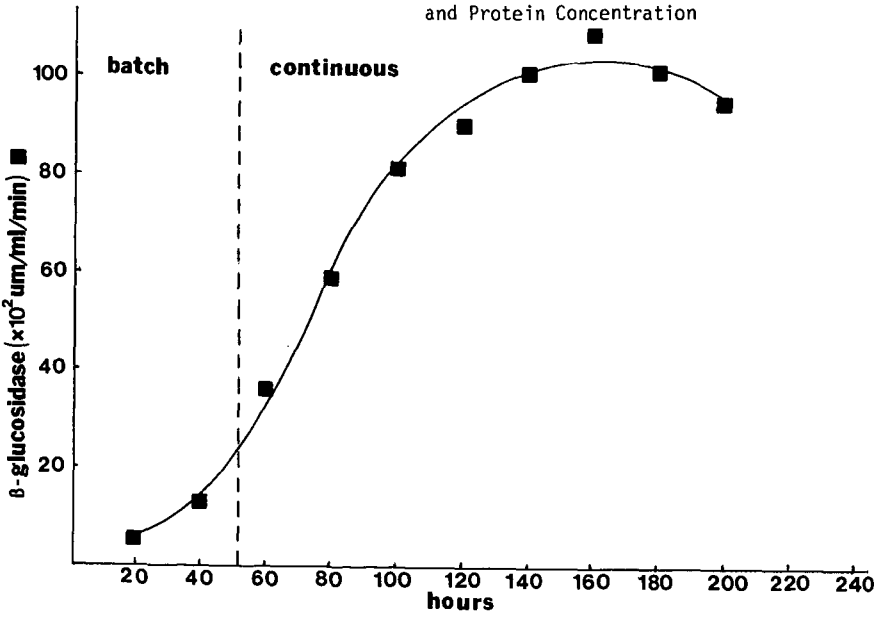

Figure 5. Continuous Enzyme Production β-*glucosidase* Activity

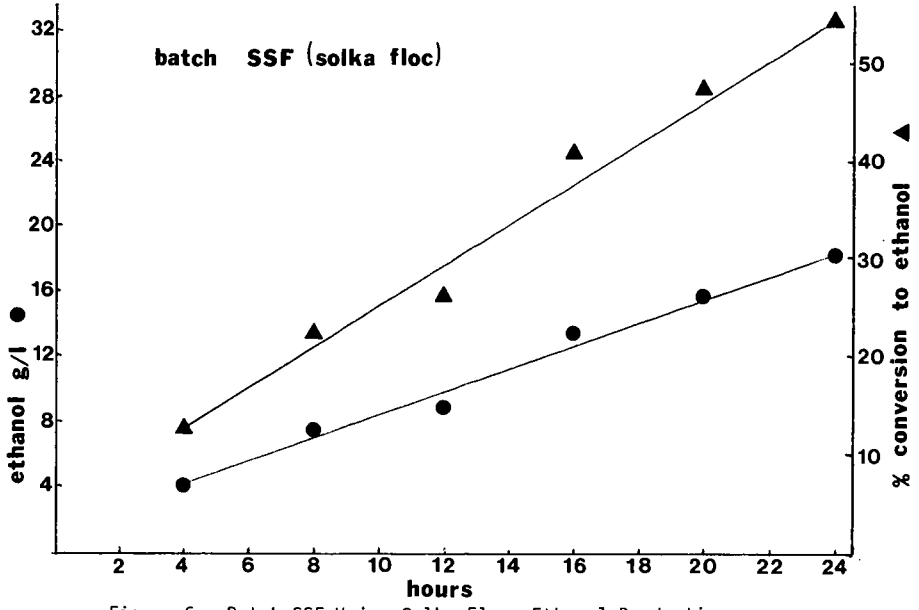

Figure 6. Batch SSF Using Solka Floc. Ethanol Production and % Conversion to Ethanol (6% Cellulose)

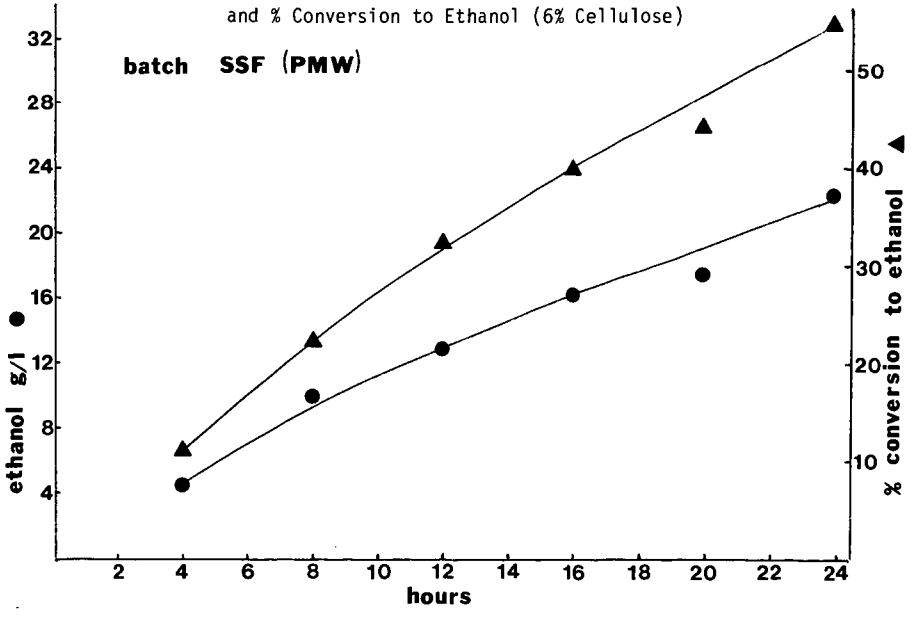

Figure 7. Batch SSF Using Pulp Mill Wastes, Ethanol Production and % Conversion to Ethanol (7% Cellulose)

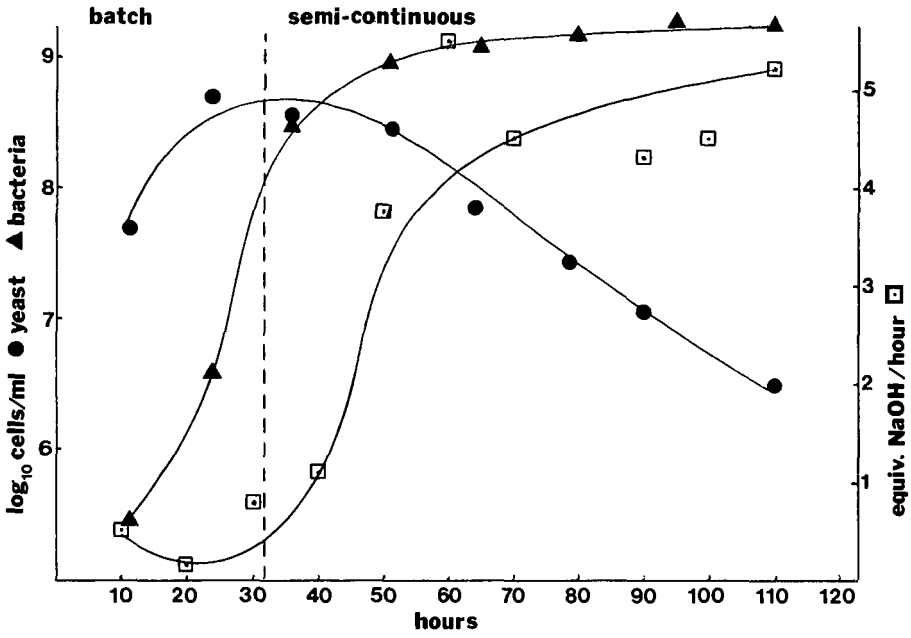

Figure 8. Semi-continuous SSF Using Pulp Mill Waste or Municipal Solid Waste, Ethanol Production (8% Cellulose)

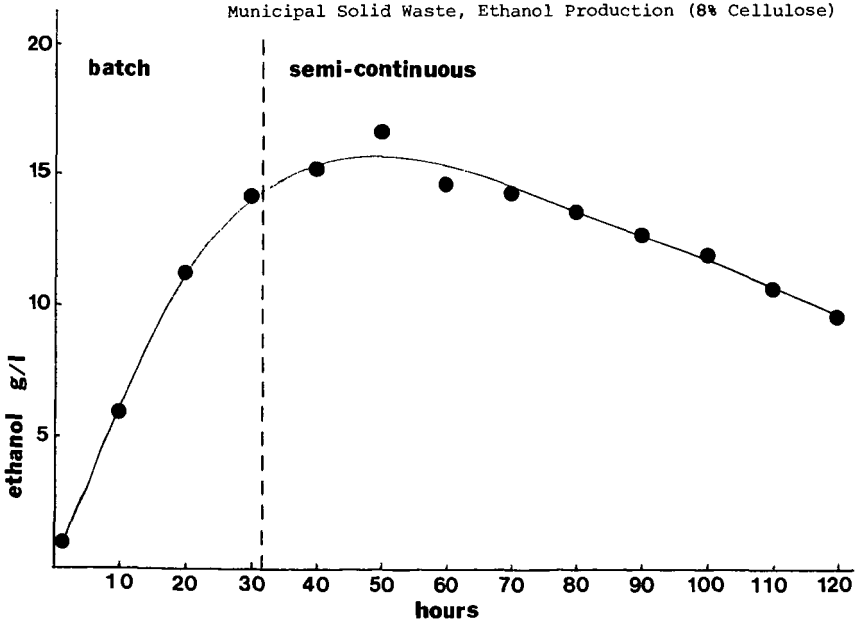

Figure 9. Semi-continuous SSF Using Pulp Mill Wastes or Municipal Solid Waste, Yeast Cell Count, Bacterial Cell Count and Base Utilization